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# ASSESSMENT OF NUTRITIONAL QUALITY AND ANTI-NUTRIENT COMPOSITION OF TWO EDIBLE GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE) - A SEARCH FOR NEW FOOD ALTERNATIVE

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#### **ABSTRACT**

Edible insects are a natural renewable resource of food that could solve the problem of food scarcity. The level of some nutrients and anti-nutrients of two grasshoppers were determined in order to ascertain their suitability as a food and feed source. *Spathosternum prasiniferum prasiniferum* contained the highest crude protein content of 65.15% while *Chrotogonus trachypterus trachypterus* had the lowest value of 59.63%. Crude fat and crude fibre content was highest in *C. trachypterus trachypterus*. Compared with the amino acid profile recommended by FAO/WHO, the grasshopper protein of studies species were of high quality due to its high content of essential amino acids. In fatty acid profile lenolenic acid was the most abundant followed by lenoleic acid. Moisture and energy contents were significantly higher in *S. prasiniferum prasiniferum*. A higher value of ash content was recorded in *S. prasiniferum prasiniferum*, corresponding to contain high proportions of sodium, potassium, calcium, phosphorus and magnesium and iron. *S. prasiniferum prasiniferum* gave higher value for Retinol, Thiamine, Riboflavin and Niacin. The anti-nutrients of the two studied species were generally low and far below the toxic level of human.Both the grasshoppers could serve as an alternative source of nutrient supplements in human diet.

**KEYWORDS**: Grasshoppers, Amino Acid, Fatty Acid, Mineral Elements, Vitamins, Anti-Nutrients

## INTRODUCTION

The deficiency of food resources has become an important issue for modern civilization and most of the developing countries are facing difficulties in providing sufficient food for their population, and thus insufficient intake of protein sometimes cause protein-energy malnutrition (Aylward and Morgans, 1995). In behalf of mankind, it is being very important to explore and exploit new nonconventional, natural and sustainable food resources. According to Aletor, (1995) insects might be such resource as they have played an important role in the history of human nutrition. Furthermore insects naturally produce a good biomass as they are an exceptionally productive group in the animal kingdom, constituting about 76% of known species of surviving animals (Yoloye, 1988), and has one trillion kg of gross weight in the world. Verkerk et al. (2007) showed that insect protein consumption could replace nearly 50% of red bovine meat consumption in the next decades. It has been found that most part of the developing world like Middle East, Latin America, Africa, Asia and Australia, consider insects as common food source (Aletor, 1995; Mitsuhashi, 1997; YhoungAree et al., 1997; De Foliart, 1999). According to Illgner and Nel (2000) throughout the world more than 1000 species of edible insects serve as traditional foods for man. Among them caterpillars, grasshoppers, beetle grubs, termites, wasps, bees, ant broods (larvae and pupae), winged ants, cicadas, and a variety of aquatic insects are some important edible insect groups (Banjo et al., 2006). Cerritos (2009) reported that a total of 320 insect species are consumed in America, 291 in Asia, 264 in Africa, 177 in Mexico and 100 in Australia. It has been reported that in South-Central Asia, the tribal community of India,

Nepal, Pakistan and Sri Lanka use 52 edible insect species as their food (Bhattacharjee, 1990). Therefore, members of class Insecta play an important role in human food resource (De Foliart, 1992; Adedire and Aiyesanmi, 1999). Their protein content range from 15% to 81% (dry basis) and these are of good quality and high digestibility (Ramos-Elorduy et al., 1997). According to Ozimek et al. (1985) insect protein concentrates have even been compared to that of milk protein. Lysine and threonine that are generally deficient in cereals, found to be high in insects. Fat, vitamin and mineral contents are also present in good amounts in edible insects (Akinnawo and Ketiku, 2000; Banjo et al., 2006).

Though different insect species had different fatty acid profiles, generally edible insects contained good quality fatty acid especially long chain omega-3 fatty acids like linolenic acid (Yang et al., 2006). Therefore, insects are recognized as valuable source of alternative nonconventional animal protein for poor, as meat from domesticated animals is scarce and beyond their economic level (Mwizenge, 1993). Phytic acid, a predominant anti-nutrient is commonly found in all insects can be easily detoxified during processing like – frying, boiling and roasting (Ekop, 2004).

A very high growth rate (Uvarov, 1966; Scoggan and Brusven, 1972; Muralirangan, 1977; Ananthakrishnan, 1986), rapid life cycle (Ananthakrishnan, 1985; Henry, 1985) and a high reproductive potential (Haldar et al., 1999; Lomer et al., 2001) help grasshoppers to produce a significant biomass in nature (Anand et al., 2008). Ramos-Elorduy and other co-workers (1982, 1984) reported that grasshoppers have a good quality protein of 52.1% to 77.1%, and their amino acid contents are higher than that of the Food and Agriculture Organization standard. Nowadays to control grasshoppers a huge quantity of toxic chemicals are used. Therefore, tons of edible insect protein is being wasted. In this context Ledger (1987) correctly pointed out that harvest method of grasshoppers for entomophagy will be much more eco-friendly. These insect pests are taken as food by several ethnic groups (Ramos Elorduy and Pino, 1993). Many grasshopper species are preserved and stored for future consumption like locusts in Africa and *Sphenarium* in Mexico (Anand et al., 2008). Attractive delicious food dishes are usually prepared from different species of grasshopper in several countries throughout the world, but nutrient analysis and compositions of many grasshopper species is yet to be evaluated. Such data would be much helpful for food consumption studies, in updating food composition tables and in diet therapy. The thrust of the present work is to determine nutritional composition, including amino acid and fatty acid profile and anti-nutrient contents of two selected multivoltine grasshoppers: *Chrotogonus trachypterus trachypterus* (Blanchard) and *Spathosternum prasiniferum prasiniferum* (Walker).

#### **MATERIALS & METHODS**

## Sample Collection

Two grasshopper species *S. prasiniferum prasiniferum* and *C. trachypterus trachypterus* were collected from nearby agricultural and grassland fields of Santiniketan (23°39′ N, 87°42′ E) India, using insect net of 30 cm diameter and sent to the Orthoptera section of the Zoological survey of India, Kolkata for taxonomic identification. The total numbers of collected individuals were 886 for *S. prasiniferum prasiniferum* and 825 for *C. trachypterus trachypterus* that were transported to the insectaria of the Entomology Research Unit, Dept. of Zoology, Visva-Bharati University for sample preparation.

## Sample Preparation

The specimens were oven-dried at 60-70°C for 72 hrs. Then the dried specimens were mechanically ground into powder and sieved through a 60-mesh screen. Before analysis the powder was stored at room temperature (18-24 °C).

#### **Proximate Composition**

Crude fat, crude fibre, ash and moisture contents were assayed by the Association of the Official Analytical Chemists (AOAC, 2006) methods. Nitrogen content was estimated by the AOAC Kjeldahl method. Crude protein content was subsequently calculated by multiplying the nitrogen content by a factor of 6.25. For the determination of carbohydrate content, grasshopper tissue was homogenized with 0.1 M phosphate buffer (pH-7) and centrifuged at 7000 g for 15 min. Then the supernatant was analysed to measure carbohydrate content following Umbreit et al. (1958) method.

#### **Energy Content**

Energy content of the specimens was estimated by Oxygen Bomb Calorimeter (Instrumentation India Co.). 1g of sample were converted into pellets by a pelletizer, charged with O<sub>2</sub> at 300 kg cm<sup>-2</sup> of pressure within the bomb and analyzed for energy content by recording the temperature rise in °C as obtained by firing the charged bomb with sample in the Digital Oxygen Bomb calorimeter.

#### **Amino Acid Analysis**

For the determination of amino acid profile of the sample PICO.TAG method was employed according to Deng et al., 2004. The amino acids determination was done from standard curves on the basis of peak area measurements. Then for all the amino acids seven-point standard curves were prepared using reference materials. Moreover, intraday variability (R.S.D.) and spike recovery studies were done. Hydrolysis of 30 mg samples with 6 M hydrochloric acid at 110°C for 24 h was performed in evacuated sealed ampoules. Then 10 ml of internal standard (α-amino butyl acid) were added mixed. After derivatisation, 100 ml PICO.TAG diluent were added and mixed. 100 ml samples were used in HPLC and analysed with PICO.TAG amino acid analyser. Basic hydrolysis method was used for the estimation of tryptophan content (Deng et al., 2004). Samples (weight equivalent to 1-2 mg tryptophan) were treated with 100ml of 4.2 M NaOH and 0.3 ml thioglycerin and kept in the oven at 110°C for a day. After cooling, the pH was adjusted to 4.5, and the mixture was made up to a certain volume. Tryptophan content was measured by colorimeter at 440 nm, where the pH was maintained at about 5.5, column temperature at 55°C, reactor temperature at 100°C, reaction time 10 to 15 min.

## Fatty Acid Analysis

Fatty acid analysis was done followed by Yang and Li (2006). 2 g of samples were extracted with 20 ml of chloroform—methanol (2:1, v/v) containing 10 mg/l of butylated hydroxyl toluene (BHT) and 0.9 mg/ml of C19:0 (nonadecanoic acid) as internal standard. Then, the mixture was stored in the fume hood for a day, then filtered and transferred to a separating funnel and added with 0.9% of 10 ml NaCl. After shaking, the phases were allowed to separate and the separated lower phase was then concentrated at 38°C and transferred to a 10 ml volumetric flask and made up to volume with chloroform containing 10 mg/l of BHT. The total oil content was determined by evaporating off the solvent at 38°C under N<sub>2</sub> to constant weight. The fatty acid methyl esters (FAMEs) of the total lipid extract were prepared by transesterification using 0.9M H<sub>2</sub>SO4 in methanol (Feng et al., 2007). Briefly, lipid solution (1 ml), 0.9M H<sub>2</sub>SO4 in methanol (3 ml) and toluene (1 ml) were added to a Teflon-capped tube and the mixture shaken strongly, then submerged in water bath at 70°C for 2 h. Then n-hexane (2 ml) and 0.9% sodium chloride (1 ml) were added to the tube and centrifuged at 1200 rpm for 15 min. The supernatant was then dropped into water (2 ml) and, after separating off the aqueous phase, dried with a little sodium sulphate anhydrous. The crude FAMEs solution obtained was filtered through a Sep-pak silica column (Alltech Associates, Inc., Deerfield, IL) before injection into the gas chromatograph, which is a Shimadzu GC-14C system equipped with a flame ionisation detector (Shimadzu Corp., Kyoto, Japan), a fused silica

capillary column (DB-23, 60 m×0.248 mm×0.25 μm: Agilent Technologies, Inc., Palo Alto, CA, USA) and the N2010 Chromatography Data System (Zhida Information Technologies, Inc., Hangzhou, China). Injection and detection temperature was 270°C and 270°C respectively. The column temperature was kept at 100°C for 3 min and programmed to 190°C at a rate of 20°C/min and kept at 190°C for 10 min. It was then increased to 205°C at a rate of 5°C/min and kept at 205°C for 6 min. Finally, it was increased to 230°C at a rate of 10°C/min and kept at 230°C for 5 min. Individual fatty acids were identified by means of purified standards (Sigma-Aldrich, Deisenhofen, Germany) and quantified by means of the internal standard method (Solver and Lanza, 1979).

#### **Mineral Content**

Firstly the samples were converted to ash by drying at 550°C. Then ash of the samples was used for minerals analysis. It was dissolved in 10% HCl (25 ml) and 5% lanthanum chloride (2 ml), boiled, filtered and made up to standard volume with distilled de-ionized water. Determination of Mg, Ca, Zn, Mn, Cu, and Fe were done by Buck Atomic Absorption Spectrophotometer (Buck Scientific Inc, Norwalk, CT, USA). Na and K were estimated with a flame photometer (Corning, Halstead Essex, UK, Model 405) (Kilgour, 1986). According to the methods of Varian Techtron (Varian, 1975) the detection limits had previously been determined. The optimum analytical range was 0.5 to 1.0 absorbance units with a coefficient of variation of 0.05 to 0.40%. Phosphovanado–molybdate method (AOAC, 2005) was followed to measure phosphorus content of the samples using a Spectronic 20 colorimeter (Gallenkamp, London, UK).

#### **Determination of Vitamins**

Thiamine (vitamin B1), riboflavin (vitamin B2) and niacin (vitamin B3) were determined by HPLC (Wimalasiri and Wills, 1985). For the estimation of Vitamin C, acetic acid and meta-phosphoric acid were used, followed by HPLC using fluorescence (Dodson et al., 1992). Alkaline saponification of the samples involved elimination of fats, liberation of natural retinol in the cells and hydrolysis of added vitamin A. Vitamin A was determined by HPLC and detected using UV and fluorescence, respectively (Manz and Philipp, 1981).

## **Anti- Nutrient Determination**

**Tannin:** Estimation of tannin content of the samples was done according to Joslyn (1970). Dried powdered samples (0.5 g) were defatted with 5% ethyl for 15 min. Tannin in the defatted sample was then extracted with methanol and the absorbance at 760 nm was measured.

**Phytic Acid:** Estimation of phytic acid content of the samples was done according to Wheeler and Ferrel (1971) using 2.0 g of the dried sample. A standard curve was made expressing the results as Fe (NO<sub>2</sub>)<sub>2</sub> equivalent.

Oxalate: This was estimated using the method of Day and Underwood (1986). To 1 g of the sample, 75 ml of 15 N H<sub>2</sub>SO<sub>4</sub> was added. The solution was carefully stirred intermittently with a magnetic stirrer for 1 h and filtered using Whatman No 1 filter paper. 25 ml of the filtrate was then collected and titrated against 0.1 N KMnO<sub>4</sub> solution till a faint pink colour appeared that persisted for 30 s.

## Statistical Analysis

Data were presented as mean  $\pm$  SD. Five replicates were carried out for all the parameters. One way analysis of varience (ANOVA) were carried out to compare the values between different grasshopper species. Duncan's multiple range tests (DMRT) were carried out for each case followed by ANOVA in order to separate the mean values according to significance. All of the analyses were carried out o using Microsoft Excel 2000 software.

# **RESULTS**

#### **Proximate Composition**

The proximate composition of *S. prasiniferum prasiniferum* and *C. trachypterus trachypterus* on a dry matter basis were summarised in Table 1. The protein content for *S. prasiniferum prasiniferum* showed significantly (df = 2; F = 129.3; P < 0.05) higher value (65.15%). Crude fat contents of the studied grasshoppers were found to be 7.15% in *S. prasiniferum prasiniferum* and 15.92% in *C. trachypterus trachypterus* which was a significantly higher (df = 2; F = 1451.2; P < 0.05) value. The moisture content of two selected grasshoppers was about 59.57% in *C. trachypterus trachypterus* and 65.91% in *S. prasiniferum prasiniferum*. Among two grasshopper species, the ash content was significantly higher (df = 2; F = 18.4; P < 0.05) in *S. prasiniferum prasiniferum*.

C. trachypterus trachypterus showed significantly higher (df = 2; F = 22.8; P < 0.05) crude fibre content. The amount of carbohydrate obtained for the grasshoppers (6.32% and 6.34% in S. prasiniferum prasiniferum and C. trachypterus trachypterus respectively) did not vary significantly (df = 2; F = 0.003; P = 0.96) between the two species. The results showed significantly higher value (df = 2; F = 21.4; P < 0.05) of nitrogen free extract for S. pr. prasiniferum.

#### **Energy Content**

The gross energy values (Table 1) for grasshoppers were appreciably high and significantly higher (23.47 KJ/g) (df = 2; F = 250.9; P < 0.05) in case of *S. prasiniferum prasiniferum*.

#### **Amino Acid Composition**

The amino acid profile of *S. prasiniferum prasiniferum* and *C. trachypterus trachypterus* is presented in Table 2. All the sulphur containing amino acids were significantly higher in *S. prasiniferum prasiniferum* (i.e. 17.77 g/100g for threonine, 0.69 g/100g for cystein and 1.78 g/100g for methionine) except valine (6.05 g/100g and 6.14g/100g in *S. prasiniferum prasiniferum* and *C. trachypterus trachypterus* respectively). Therefore, total of sulphur amino acids was significantly higher in *S. prasiniferum prasiniferum*. Isoleucine content of both the selected species showed lower results that were around 1.24 g/100g and 1.26 g/100g in *S. prasiniferum prasiniferum* and *C. trachypterus trachypterus* respectively. Among essential amino acids leucine, lysine and histidine were significantly higher in *S. prasiniferum prasiniferum* and tyrosine and phenylalanine were in *C. trachypterus trachypterus*. Total essential amino acids was found to be significantly higher (df = 4; F = 46.3; P < 0.05) in *S. prasiniferum prasiniferum* (57.81 g/100g). Among nonessential amino acids asparagine, glutamic acid, glycine and alanine were significantly higher in *S. prasiniferum prasiniferum* and serine, proline, arginine and tryptophan were in *C. trachypterus trachypterus*. But the total nonessential amino acid content did not vary significantly (df = 4; F = 2.11; P = 0.22) between the selected species that were about 39.83 g/100g in *S. prasiniferum prasiniferum* and 40.57 g/100g in *C. trachypterus trachypterus*.

## **Fatty Acid Composition**

Table 3 shows the fatty acid profile of the chosen grasshoppers. Among the fatty acids, content of unsaturated fatty acids (PUFA) were higher, especially the eicosenoic acid, linoleic acidand linolenic acid that were significantly higher in *S. prasiniferum prasiniferum* (15.5 g/100g, 29.26 g/100g and 39.45 g/100g for eicosenoic acid, linoleic acidand linolenic acid respectively) between the grasshopper species.

#### **Mineral Contents**

As shown in Table 4, the selected grasshopper species contained 9 minerals, of which Na, K, Ca, P, Mg and Zn were the most predominant. All the mineral contents were significantly higher (df = 8; F = 1626; P < 0.05) in S.

prasiniferum prasiniferum. A high amount of Na, K, Ca, Mg, P and Fe but relatively low amount of Zn, Mn and Cu were found in the grasshoppers of interest. The result showed that K had the highest concentration (2051mg/kg in *C. trachypterus trachypterus* and 2994mg/kg in *S. prasiniferum prasiniferum*) while Mn recorded the lowest value (0.22 mg/kg in *C. trachypterus trachypterus* and 0.38 mg/kg in *S. prasiniferum prasiniferum*). The next abundant mineral element was Na which ranged from 223 mg/kg to 291 mg/kg.

#### **Vitamin Contents**

In the present study five vitamins (A, B1, B2, B3 and C) were observed in both the grasshopper species (Table 5). Between the two selected grasshopper species, all the above mentioned vitamins except ascorbic acid were observed in greater amount in *S. prasiniferum prasiniferum*. The result revealed that vitamin A had the highest amount (6.2 mg/100g in *C. trachypterus trachypterus* and 6.5 mg/100g in *S. prasiniferum prasiniferum*). The next abundant was vitamin B3 which was 4.25 mg/100g in *C. trachypterus trachypterus* and 5.49 mg/100g in *S. prasiniferum prasiniferum*. Vitamin B1 was found in lowest amount (0.08 mg/100g in *C. trachypterus trachypterus trachypterus* and 0.15 mg/100g in *S. prasiniferum prasiniferum*).

#### **Anti-Nutrient Composition**

The result of anti-nutrient composition is presented in Table 6. Between the two grasshopper species, C. trachypterus trachypterus had significantly higher level of anti-nutrient contents except phytin which did not vary significantly (df = 4; F = 0.006; P = 0.94). Among the detected anti-nutrients tannin content was higher (4 g/100g in C. trachypterus and 1.1 g/100g in C. trachypterus and 1.1 g/100g in C. trachypterus and 0.014 g/100g in C. trachypterus trachypterus and 0.014 g/100g in C. trachypterus trachypterus and 0.014 g/100g in C.

# **DISCUSSIONS**

#### **Proximate Composition**

The results of protein content of studied acridids (59.63% and 65.15%) corroborated and compared favourably with *Acrida cineria* reported by Wang et al. (2007). The values of the studied grasshoppers were also found to be higher than 49.87% found in *Zonocerus variegatus* (Ekop et al., 2010), 22.12% in many grasshopper species (Adedutan, 2005) and the values of three grasshopper species reported by Banjo et al. (2006) but lower than 74% for *Melanoplus bivittatus* (McHargue, 1917), 71.3% for *Melanoplus mexicanus* (Ueckert et al., 1972) and the 77% for *Sphenarium histrio* (Ramos-Elorduy, 1997). Jokthan et al. (2007) reported that grasshoppers have significantly higher crude protein content than some micro-livestock (snail, Cricket, Termite and Earthworm). Generally, insects were good sources of protein content as high as 82% has been reported in the edible insect wasp (Polybia sp.) (Ramos-Elorduy et al., 1997). The high protein content was an indication that the studied grasshoppers could be valuable for man and could equally replace higher animal protein usually absent in the diet of rural dwellers in most developing and under-developed countries. So, insect protein could contribute daily protein requirement of human as recommended by NRC (1980).

The fat content of *S. prasiniferum prasiniferum* was almost similar with the value of *Oxya fuscovittata, Acrida exaltata, Hieroglyphus banian, Melanoplus mexicanus* and *Acrida cineria* respectively (Ramos-Elorduy et al., 1997; Wang et al., 2007; Anand et al., 2008). These values were higher than the same of some other grasshoppers found in literature (Ramos-Elorduy et al., 1997; Banjo et al., 2006; Adeyeye and Awokunmi 2010). Fat are essential in daily human diets as they increase the palatability of foods by absorbing and retaining their flavours (Aiyesanmi and Oguntokun, 1996). These are also vital in the structural and biological functioning of the cells and help in the transport of nutritionally essential fat-soluble vitamins (Omotoso, 2006). The fat content of studied grasshoppers in the present investigation represented a good source of oil though it was lower than the fat content of *Agrotis ipsilon* (53.1%) reported by Ghaly (2009).

The moisture content of two selected grasshoppers was comparable with the values reported in other edible insects like *Tenebrio molitor* (61.5%) (Ghaly and Alkoaik, 2009) and white grubs (64.01%) (Alhassan et al., 2009). As these grasshoppers are used as food only after drying one may conclude that both the studied grasshoppers could be preserved for a reasonable period of time without the risk of microbial deterioration and spoilage. A long "shelf-life" is an added advantage over other sources of protein like beef, egg, fish, which are easily prone to spoilage on careless keeping (Ekop et al., 2010).

The percentages of ash content of the chosen grasshoppers were lower than some edible Orthoptera species i.e. *Brachytrypes spp.* (1.82%), *Cyrtacanthacris aeruginosus unicolor* (2.10%) and *Zonocerus variegatus* (1.20) (Banjo et al., 2006). Mineral contents of a sample could be assumed by its ash content (Omotoso, 2006).

Higher crude fibre content of *C. trachypterus trachypterus* could be attributed to little amount of chitin found normally in insects (Akinnawo and Ketiku, 2000). "The physiological role of crude fibre in the body is to maintain an internal distension for proper peristaltic movement of the intestinal tract" (Oduor et al., 2008). "A diet very low in fibre, could therefore lead to constipation which might bring discomfort to the body system with running stool" (Groff et al., 1999). "Diets with high fibre content have been used for weight control and fat reduction, as they give a sense of satiety even when small food is eaten" (Ekop, 2004). Crude fibre content varied widely with literature surveyed data for different species of insects might be due to different species had different exoskeletons and structure. The results of crude fibre content (6.91-7.89%) of the selected grasshoppers were higher than those of Banjo et al. (2006) for Orthoptera. This proves that the chosen grasshopper species are good sources of crude fibre.

The values of carbohydrate content are slightly higher than literature (Dunkel, 1996) value for grasshopper (2.2%). Though the entire values of carbohydrate were enough low for human need. So they are therefore not a good source of carbohydrate as human adult need about 400 - 500 g carbohydrate intake as starch. "But according to last meeting of the American Heart Association, has been focused on low-carbohydrate—high protein (LC—HP) diets run counter to all the current evidence-based dietary recommendations for healthy populations. It has been suggested that these diets, which were introduced originally as weight-loss regimens, also have a significantly beneficial effect on a variety of cardiovascular risk factors. It is clear that people who consume such diets have a reduced intake of calories, resulting in a predictable degree of weight loss." (Kappagoda et al., 2004).

The present investigation supports the view of Ramos-Elorduy (1997) and Bukkens (1997) that grasshoppers have appreciable food value as their proximate composition is better than the conventional food like beef, lamb, pork, chicken, fish, milk and egg. Moreover grasshoppers also have favourable nutrient contents, when compared with other edible insects (Bukkens, 1997; Ramos-Elorduy, 1997).

# **Energy Content**

The values of energy content of the two studied grasshoppers were much higher than some other orthopterans like *Brachytrypes membranaceus* (Adeyeye and Awokunmi, 2010). Hence, studied grasshoppers could contribute greatly to the calorie content of food.

#### **Amino Acid Composition**

The studied grasshoppers have higher essential and nonessential amino acid contents than that of *Acrida cineraia* and fish (Wang et al., 2007). Cystein content of *Acrida cineraia* was similar to the value of *S. prasiniferum prasiniferum*. *C. trachypterus trachypterus* had lower amounts of sulphur amino acids than *Acrida cineraia* and fish meal. Fish meal had

slightly higher lysine content than *C. trachypterus trachypterus*. Tryptophan and lysine deficiency were observed in some grasshoppers (Ramos-Elorduy, 1997), though in the present study these were not the limiting amino acids. Moreover, studied grasshoppers have adequate amino acid profile that meets the highly demanding preschooler W.H.O requirements for all except sulphur amino acids, isoleucine and leucine. The high lysine content of *S. prasiniferum prasiniferum* can help offset cereal-based diets which are lysine- deficient. Threonine is the limiting amino acids in wheat, rice, cassava and maize based diets that are prevalent in the developing world (Hill, 1970; Ozimek et al., 1985), while leucine and histidine have been reported to enhance the growth of infants and young children (Cameron and Hofvander, 1980). These amino acids were present in adequate proportion in both the studied grasshopper species. Therefore, it could be stated that the studied grasshopper proteins contain all the amino acids needed by humans in the almost right proportions. According to Qian (1997) grasshoppers are the source of high quality protein that could be used for human nutrition and the present study also support this opinion.

#### **Fatty Acid Composition**

Among the fatty acids, substantial amounts of PUFA, especially the eicosenoic acid, linoleic acid and linolenic acid indicated that the chosen grasshoppers should be given attention as a potential oil source for food industry. According to Ekop and Onigbinde (2005), the level of unsaturation observed in grasshopper fat content is higher than what is obtainable in most animal lipids, as well as for palm oil and coconut oil, which are common household oils. In this context, DeFoliart (1991) also reported that the insect fatty acids are similar to some of the conventional food like poultry and fish in their degree of unsaturation. Unsaturated fatty acids play an important role to prevent coronary heart diseases, hypertension and diabetes in human (Haglund, 1998), so these have a prospect of medicine. Palmitic acid was found to be higher in *Acrida cineria* than *S. prasiniferum prasiniferum*. It's higher amount raise low density lipoprotein (LDP) cholesterol and therefore considered atherogenic (Willett and Sacks, 1991). *C. trachypterus trachypterus* gave higher value of stearic acid that has been shown not to raise plasma cholesterol (Bonamone and Grundy, 1987; NRC, 1988). In insects, cellular responses are modulated by prostaglandins (PGs) that are products of eicosenoic acid (Raksakantong et al., 2009). This fatty acid is also present in sufficient amount in *S. prasiniferum prasiniferum*. Therefore, the present study proves that the chosen grasshoppers contain good quality fatty acids.

# **Mineral Contents**

Mineral contents of both the species were within acceptable level. Previous reports (Anand et al., 2008; Banjo et al., 2006) identified 6 minerals in four different grasshopper species and also 9 minerals in *Brachytrypes membranaceus*. In the latter one, all the minerals were present in lower quantity than the studied grasshopper except Zn content that was lower in selected grasshoppers. The amount of Ca, Mg, Fe and P of some grasshopper species studied by Banjo et al. (2006) and Adedutan (2005) were lower than the grasshoppers of the present study. Calcium is the fifth most common element in the body. Mc Donald et al. (1995) reported that intracellular Ca functions in muscle contraction, hormone secretion, glycogen metabolism and cell division, and the extracellular Ca provides ions for the maintenance of intracellular Ca, bone mineralization, blood coagulation, and plasma membrane potential. According to Greenwood (2011) magnesium is needed for more than 300 biochemical reactions in the body. It helps maintain normal muscle and nerve function, supports a healthy immune blood and regulates blood sugar levels (Saris et al., 2000), prevents cardiomyopathy (Chaturvedi et al., 2004) and regulates acid-alkaline balance (Fallon and Enig, 1999). The iron content in *S. prasiniferum prasiniferum* (7.2 mg/kg) was higher than some fresh water fishes (2-5 mg/kg) (Adeyeye, 1993; Adeyeye, 1996) and might be favourably compared with termites (7.5 mg/kg) (Lokeshwari and Shantibala, 2010). Therefore, *S. prasiniferum prasiniferum* may solve the problem of iron deficiency of pregnant women's diets in the developing world, especially in

Africa as reported by Orr (1986). Trace elements (Zn and Cu) present in the studied grasshoppers were necessary for physiological functions (Liu, 1996) and has an important biological role in enzyme systems (Halberge et al., 1993; Lee, 1996; Harrison and deMora, 1996). The ratio of sodium and potassium in both the species of grasshopper were approximately 1:10. Grasshoppers with this favourable ratio may prevent hypertension as potassium intake has been found to lower blood pressure by antagonizing the biological effect of sodium (Einhorn and Landsberg, 1988). The potassium contents were far greater than the values of 450 mg/kg in *Z. variegatus* and 125–169 mg/kg in the Nigerian freshwater fish (Adeyeye et al., 1996). The values of Na and K in the selected grasshoppers were also better than the values in *Illisha africana* fish which contained 62–148 and 64–87 mg/kg of Na and K respectively (Adeyeye, 1996). According to Malik and Srivastava (1982) potassium is an essential nutrient and has an important role is the synthesis of amino acids and proteins. Whereas sodium in addition to neutralizing acid and activating body fluid and organs is an important component for muscle and nerve functions as well as for the proper functioning of the liver, pancreas, and gall bladder.

Thus it could be said that daily requirements of essential minerals could be derived from the consumption of these studied grasshoppers especially the *S. prasiniferum prasiniferum*.

#### **Vitamin Contents**

Various authors reported that the edible insects contain vitamins like A, B1, B2, B3, B6, D, E, K and C (DeFoliart 1991; Lu et al. 1992; Chen and Feng 1999; He et al. 1999; Feng et al. 1999, 2000a, b; 2001a, b, c). Some grasshoppers like *Brachytrypes sp*, *Cytacanthacris aeruginosus unicolor* and *Zonocerus variegatus* showed the presence of Vitamin A, B2 and ascorbic acid (Banjo et al., 2006). In the present case the amount of vitamins A and B2 were higher in the studied grasshopper species, whereas ascorbic acid was present in the amount which was much lower than some other grasshoppers like *Zonocerus variegatus*. Edible stink bug have less amount of Vitamin A, B1, and B2 than studied species (Teffo, 2007). Vitamin B1, B2 and B3 were found to be present in higher amount in the studied grasshopper species than termites, and their contents are lower than weevil, but favourably comparable with caterpillar (Lokeshwari and Shantibala, 2010).

#### **Anti-Nutrient Composition**

The tannin contents of both the grasshopper species recorded were within the acceptable range. Aletor (1995) reported that high level of tannins (7.6 to 9.0 g/100g of dry matter) could be detrimental if consumed as tannins usually form insoluble complexes with protein thereby interfering with their bioavailability and high tannin in diets is ascribed to its astringent property, which is a consequence of its ability to bind with proteins of saliva and mucosal membranes. The phytin contents were lower than values of boiled and fried locust flour, and *P. chilensis* (Nafisa et al., 2008; Vijayakumari et al., 1997 respectively). According to Oke (1969), a phytate diet of 1 to 6% decreases the bioavailability of mineral elements in mono gastric animals as it binds to mineral elements such as calcium, zinc, manganese, iron and magnesium to form complexes that are indigestible, thereby decreasing their bioavailability of these elements for absorption (Erdman, 1979). The result obtained in this study indicated that the range of phytin and phytin P in studied grasshoppers could not pose health risk. The oxalate level (0.51 g/100g in *S. prasiniferum prasiniferum* and 0.68 g/100g in *C. trachypterus trachypterus*) recorded in this work is lower than the value reported by Omotoso, (2005) for *C. armatum*. According to Ladeji et al. (2004), oxalate can bind to calcium present in food thereby rendering calcium unavailable for normal physiological and biochemical functions. Thus both the studied grasshopper species contain much lower amount of all the studied anti-nutrients and so might be recommended for consumption.

The values obtained from the proximate analysis of the chosen grasshoppers, generally were in concert with most reports by other authors investigating different edible insects from several parts of the world. Thus, they are not just a traditional food but contain reasonable level of protein, fat, especially essential amino acids, unsaturated fatty acids, vitamins and minerals. The anti-nutrient contents of both the species studied, were observed to be generally low and within safe consumption levels. Therefore, the preliminary results presented in this paper confirmed that these grasshoppers especially *S. prasiniferum prasiniferum* could be a potential food and might be used to the food industry as a source of ingredients with high nutritional value. They have potentials of increasing the protein intake of low to average income earners or poor sections of populations throughout the developing countries to alleviate the problem of nutrient/protein malnutrition. It is also considered that grasshoppers can be used as future food to resolve global food shortages. Furthermore, detailed analysis of these selected grasshoppers for other nutrients, anti-nutrients and secondary metabolites with medicinal potential should be undertaken. Not only that, studies of more grasshopper species should be needed to broaden animal protein resource base. Mass rearing of suitable grasshopper species with modern techniques would increase their commercial values and availability and decrease the pressure exerted on the conventional sources of proteins.

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# APPENDICES

Table 1: Proximate Analysis and Energy Content of Studied Grasshoppers

Nutritional Composition (%)	Spathosternum prasiniferum prasiniferum	Chrotogonus trachypterus trachypterus
Protein	65.15±0.41b	59.63±0.26a
Crude fat	7.15±0.19a	15.92±0.13b
Crude fibre	6.91±0.14a	7.89±0.15b
Carbohydrate	6.32±0.36a	6.34±0.18a
Ash	11.07±0.11b	8.55±0.02a
Moisture	65.91±0.24b	59.57±0.18a
Energy (KJ/g)	23.47 ±1.54b	16.93±0.99a

<sup>&</sup>lt;sup>a</sup>Mean± SD. Within a row a, b indicates significant differences between mean values. (One way ANOVA, DMRT, P<0.0001)

Table 2: Amino Acid Composition (G/100 G) of Studied Grasshoppers

Amino Acid (G/100 G of Protein)	Spathosternum prasiniferum prasiniferum	Chrotogonus trachypterus trachypterus	WHO/FAO /UNU* Preschooler	WHO/FAO /UNU* Adult
Threonine	17.77±0.56b	15.5±0.09a		
Cystein	0.69±0.03b	0.43±0.02a		
Valine	6.05±0.02a	6.14±0.03b	3.5	1.3
Methionine	1.78±0.01b	1.24±0.02a		
Total of sulphur amino acids	2.47±0.04b	1.67±0.04a	2.5	1.7
Isoleucine	1.24±0.01a	1.26±0.01a	2.8	1.3
Leucine	5.13±0.01b	5.07±0.01a	6.6	1.9
Tyrosine	9.65±0.06a	11.52±0.11b	6.3	1.9
Phenylalanine	4.16±0.01a	4.96±0.05b		
Lysine	5.95±0.02b	3.04±0.09a	5.8	1.6
Histidine	5.39±0.04b	4.69±0.22a	1.9	1.6
Total essential amino acids	57.81±0.77b	53.85±0.65a		
Asparagine	0.47±0.01b	0.00±0.00a		
Serine	4.55±0.01a	5.04±0.04b		
Glutamic acid	3.75±0.02b	3.06±0.01a		
Proline	15.93±0.05a	16.96±0.08b		
Glycine	8.4±0.13b	7.94±0.15a		

Table 2: Contd.,				
Alanine	3.15±0.02b	2.85±0.05a		
Arginine	7.25±0.02a	8.3±0.04b		
Tryptophan	2.33±0.01a	2.42±0.01b		
Total non- essential amino acids	39.83±0.27a	40.57±0.75a		

<sup>&</sup>lt;sup>a</sup>Mean± SD. Within a row a, b indicates significant differences between mean values within grasshopper species. (One way ANOVA, DMRT, P<0.001)

Table 3: Fatty Acid Composition (G/100 G) of Two Grasshoppers

Name of Fatty Acid (G/100 G of Protein)	Spathosternum prasiniferum prasiniferum	Chrotogonus trachypterus trachypterus
Myristic acid	1.16±0.02a	5.76±0.01b
Palmetic acid	1.05±0.11a	18.67±1.25b
Palmitoleic acid	2.58±0.05b	2.11±0.02a
Oleic acid	7.25±0.96b	2.01±0.01a
Stearic acid	1.55±0.02a	7.21±0.01b
Eicosenic acid	15.5±0.85b	3.91±0.01a
Lauric acid	0.39±0.03a	1.78±0.07b
Linoleic acid	29.26±1.10b	14.79±0.88a
Linolenic acid	39.45±1.10b	18.01±0.88a

<sup>&</sup>lt;sup>a</sup>Mean±SD. Within a row a, b indicates significant differences between mean values within grasshopper species. (One way ANOVA, DMRT, P<0.001)

Table 4: Mineral Composition (Mg/Kg) of Two Grasshoppers

Names of Minerals	Spathosternum prasiniferum	Chrotogonus trachypterus
(Mg/Kg)	prasiniferum	trachypterus
Sodium	291.90±2.20b	223.2±2.6a
Potassium	2994.00±1.51b	2051.00±1.44a
Calcium	11.64±0.32b	5.2±0.22a
Magnesium	7.95±0.02b	6.7±0.04a
Phosphorus	8.76±0.06b	5.77±0.04a
Zinc	3.81±0.08b	3.02±0.05a
Iron	7.20±0.07b	2.1±0.09a
Manganese	0.38±0.01b	0.22±0.01a
Copper	2.21±0.04b	2.07±0.05a

<sup>&</sup>lt;sup>a</sup>Mean±SD. Within a row a, b indicates significant differences between mean values within grasshopper species. (One way ANOVA, DMRT, P<0.001)

Table 5: Vitamin Contents (G/100 G) of Two Grasshoppers

Names of Vitamin (Mg/100g)	Spathosternum prasiniferum prasiniferum	Chrotogonus trachypterus trachypterus
Retinol (Vitamin A)	6.50±0.05b	6.20±0.03a
Thiamine (Vitamin B1)	0.15±0.01b	0.08±0.01a
Riboflavin (Vitamin B2)	1.30±0.02b	1.00±0.01a
Niacin (Vitamin B3)	5.49±0.09b	4.25±0.03a
Ascorbic acid (Vitamin C)	4.66±0.06a	6.20±0.04b

<sup>&</sup>lt;sup>a</sup>Mean±SD. Within a row a, b indicates significant differences between mean values within grasshopper species. (One way ANOVA, DMRT, P<0.001)

Table 6: Anti-Nutrient Composition ( $G/100\ G$ ) of Two Grasshoppers

Name of Anti- Nutrient (G/100 G)	Spathosternum prasiniferum prasiniferum	Chrotogonus trachypterus trachypterus
Oxalate	0.510±0.020a	0.680±0.030b
Tannin	1.100±0.040a	4.000±0.220b
Phytin	0.066±0.010a	0.067±0.020a
Phytin P	0.014±0.000a	0.018±0.0001b

Phytin P | 0.014±0.000a | 0.018±0.0001b | aMean±SD. Within a row a, b indicates significant differences between mean values within grasshopper species. (One way ANOVA, DMRT, P<0.001)